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SOIL ACTIVATOR AND ITS METHOD OF MANUFACTURE  
[DOJO KASSEIZAI OYABI SONO SEIZO HOHO]

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## 1. Title of the Invention

Soil Activator and Its Method of Manufacture

## 2. Claim(s)

(1) A soil activator characterized by being comprised by making an inoculum from microorganisms, such as thermophilic fibrinolytic bacteria, Actinomycetes, filamentous bacteria, yeast fungi, photosynthetic bacteria, or heterotrophic bacteria, which are useful for decomposing and humifying of organic substances in the soil, culturing them on a culture medium, and specific organic nitrogen sources, vitamins, minor nutrients, minor growth factors needed by these microorganisms being adsorbed on a mixture of vermiculite and calcium carbonate rock powder.

(2) A method for manufacturing a soil activator characterized by making an inoculum from microorganisms, such as thermophilic fibrinolytic bacteria, Actinomycetes, filamentous bacteria, yeast fungi, photosynthetic bacteria, or heterotrophic bacteria, which are useful for decomposing and humifying organic substances in soil using peptone, excess calcium carbonate, ammonium and sodium hydrogen phosphate, potassium dihydrogen phosphate, magnesium sulfate, calcium chloride, ferric chloride, fibrin, and water, such as well water, tap water or other clean water, in a Viljoen, Fred, Peterson (1926) culture medium, or natural culture medium, then culturing this for 48 to 60 hours under anaerobic or facultative anaerobic conditions at  $60\pm5^{\circ}\text{C}$ , adding vermiculite and calcium carbonate rock powder to this, and stirring this well to mix and adsorb it thereon.

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\* Numbers in the margin indicate pagination in the foreign text.

(3) A method for manufacturing a soil activator characterized by making an inoculum from microorganisms, such as thermophilic fibrinolytic bacteria, Actinomycetes, filamentous bacteria, yeast fungi, photosynthetic bacteria, or heterotrophic bacteria, which are useful for decomposing and humifying organic substances in soil using peptone, excess calcium carbonate, ammonium and sodium hydrogen phosphate, potassium dihydrogen phosphate, magnesium sulfate, calcium chloride, ferric chloride, fibrin, and water, such as well water, tap water or other clean water, in a Viljoen, Fred, Peterson (1926) culture medium, or natural culture medium, then culturing this for 48 to 60 hours under anaerobic or facultative anaerobic conditions at  $60\pm5^{\circ}\text{C}$ , adding vermiculite and calcium carbonate rock /168 powder to this, stirring this well to mix and adsorb it thereon, and shaping this into a suitable form, such as a granular form.

### 3. Detailed Specifications

This invention pertains to improving a soil activator and its method of manufacture, by (1) making an inoculum from microorganisms, such as thermophilic fibrinolytic bacteria, Actinomycetes, filamentous bacteria, yeast fungi, photosynthetic bacteria, or heterotrophic bacteria, which are useful for decomposing and humifying of organic substances in the soil, culturing them on a culture medium, and specific organic nitrogen sources, vitamins, minor nutrients, minor growth factors needed by these microorganisms being adsorbed on a mixture of vermiculite and calcium carbonate rock powder, (2) by making an inoculum from microorganisms, such as thermophilic fibrinolytic bacteria, Actinomycetes, filamentous bacteria, yeast fungi, photosynthetic bacteria, or heterotrophic bacteria,

which are useful for decomposing and humifying organic substances in soil using peptone, excess calcium carbonate, ammonium and sodium hydrogen phosphate, potassium dihydrogen phosphate, magnesium sulfate, calcium chloride, ferric chloride, fibrin, and water, such as well water, tap water or other clean water, in a Viljoen, Fred, Peterson (1926) culture medium, or natural culture medium, then culturing this for 48 to 60 hours under anaerobic or facultative anaerobic conditions at  $60\pm 5^{\circ}\text{C}$ , adding vermiculite and calcium carbonate rock powder to this, and stirring this well to mix and adsorb it thereon plus (3) shaping this into a suitable form, such as a granular form, and the object is to obtain a soil activator for artificially culturing bacteria which is effective on soil, such as, thermophilic fibrinolytic bacteria, Actinomycetes, yeast fungi, photosynthetic bacteria, or heterotrophic bacteria, and scattering and propagating this as an inoculum, to reliably promote the decomposition and humification of the organic substances and to plan fertilization of soil.

It is not necessary to point out yet again that Japan has few resources, and that the soil is our most important resource, and is the foundation of farming. However, besides the harsh natural conditions in Japan, such as acidification of soil due to downpours and runoff of bases, the soil in Japan is sometimes cultivation with insufficient labor due to a shortage of labor or the like while guiding the direction of high-fertilization and high-harvesting farming methods by chemical fertilizers and [illegible] performed intensely day and night, there is strong concern that the soil will become inferior, the soils resistance against weather and fire disasters

or the like also will weaken, and the soil's fertility will decrease. Intense contemplation with respect to this reality is a powerful driving force in a "movement for soil preparation" by the many concerned institutions like local jurisdictional government offices.

Soil preparation is a synthetic effort for satisfying the environment or conditions for soil for growing plants to maximize its function. A final goal of soil preparation is to stimulate the activity of microorganisms in the soil by the application and deep plowing of good-quality organic substances, and a genuine humus is accumulated in the soil as a physically-, chemically-, and biologically-stabilized substance.

The root of soil fertility is the humus therein. Humus is rich in organic nitrogen and is an extremely important agricultural substance because it promotes the adsorption and retention of cations, which are mineral ameliorants, a chelating action, the granulating of soil, and microbial activity without compromising the soil preparation; the physical and chemical properties are closely related to the activity of the microorganisms in the soil. Various personifications of soil have been implemented, such as "the soil is alive," "the soil is tired," "the soil is dead," etc.

There is an ability to change the form of the substances in the soil.

This ability is a chemical change induced by organisms, referred to as a biochemical change. This biochemical-changing ability is called soil activity. That is, soil activity is most often derived from microorganisms.

Therefore, the object of the present invention is to synthetically culture bacteria which are effective in soil, such as thermophilic fibrinolytic bacteria, Actinomycetes, yeast fungi, photosynthetic bacteria, or heterotrophic bacteria and scatter and propagate them as /169 an inoculum to accelerate the decomposition and humification of organic substances more reliably and plan richer fertilization of the soil.

Next, the constitution of this invention comprises four fundamental steps, i.e., (I) culturing facultative anaerobic or just anaerobic bacteria, such as thermophilic fibrinolytic bacteria or Rhodospirillaceae, (II) culturing anaerobic bacteria, such as filamentous bacteria, Actinomycetes, yeast fungi, or heterotrophic bacteria, (III) adding vitamins and minor growth factors as specific organic nitrogen sources, and (IV) manufacturing the soil activator of this invention by mixing the manufacture of an excipient, by mixing vermiculite and calcium carbonate rock powder, with the culturing of the aforesaid microorganism.

The specific feature of this invention that is emphasized is that a mixture of vermiculite and calcium carbonate rock powder is used as the excipient.

Vermiculite has the following superior properties.

(a) Vermiculite

Sieved vermiculite that is dried and subsequently baked around 1,000°C is referred to as regular vermiculite.

### Analysis Table of Vermiculite

SiO <sub>2</sub>	Silicic Acid	45.07%
TiO <sub>2</sub>	Titanium	1.84
Al <sub>2</sub> O <sub>3</sub>	Alumina	15.25
Fe <sub>2</sub> O <sub>3</sub>	Ferric oxide	13.17
FeO	Ferrous oxide	1.08
MgO	Magnesia	7.16
CaO	Lime	2.01
K <sub>2</sub> O	Potash	3.32
+H <sub>2</sub> O	Nonvolatile crystal water at 100°C	5.80
+H <sub>2</sub> O	Volatile moisture at 100°C	2.23
Other		3.07

The table with the above constituents is one example of vermiculite, and the potassium content of the vermiculite per se is high.

(b) Since the porosity is high, the moisture absorption and water holding capacity are excellent, and the drainage and air distribution is developed well, only sophisticated microorganisms are enriched.

(c) Since it has a remarkably powerful substitutability for bases, its fertilizer-holding ability is good, and it has a superior ability to control excess fertilizer.

For example, it exhibits a unique effect in preventing Wakatsuchi deficiency caused by excess potash.

(d) Rooting of cultured plants is vital, and if their hair roots are solidly established in it, they will penetrate into the vermiculite; hence, the plants are hurt little.

#### **Facultative anaerobic, or just aerobic culturing of thermophilic fibrinolytic bacteria**

(a) Culturing of thermophilic fibrinolytic bacteria

Fibrinolytic bacteria include various kinds of bacteria, such as Actinomycetes and filamentous bacteria. However, in terms of the vitality of the fibrinolytic ability, the wide-ranging breeding conditions, and

the like, thermophilic bacteria, such as *Crostridium Thermocellum*, *Bacillus Thermocelluloytieus*, *Bacillus Thermofibrincolus*, and *Bacillus Celulosae dissolveus*, play important roles in the decomposition and humification of vegetable organic substances.

For culturing thermophilic fibrinolytic bacteria, 5 g of peptone, excess calcium carbonate, 2 g of ammonium and sodium monohydrogen phosphate, 1 g of potassium dihydrogen phosphate, 0.3 g of magnesium sulfate, 1 g of calcium chloride, 15 g of fibrin, and 1,000 cc of well water or tap water are used for a Viljoen, Fred, Peterson (1926) culture medium. Part of this culture medium composition may be replaced with natural materials.

The bacteria are cultured for 48 to 60 hours under  $60 \pm 5$  °C facultative anaerobic conditions.

(b) Culturing *Rhodospirillaceae*

Photosynthetic bacteria are roughly classified into three types: /170 *Chlorobiceae*, *Chloroflexaceae*, and *Rhodospirillaceae*. The bacteria used mainly in this invention are *Rhodospirillaceae*. The low-molecular weight organic acids, amino acids, alcohols, and the like produced by the superior properties of these bacteria, that is, the decomposition of the organic substances, are assimilated well, hydrogen sulfide is decomposed, and the ability for fixing nitrogen from the air, and the like is put to practical use proactively.

For culturing *Rhodospirillaceae*, a Huimer (1946) culture medium, in which the following constituents were dissolved in distilled water, i.e.,  $K_2HPO_4$  0.05 (%),  $KH_2PO_4$  0.05 (%),  $(NH_4)_2HPO_4$  0.08 (%),  $MgSO_4$  0.02 (%), lactic acid 0.3 (%), acetic acid 0.1 (%), citric acid 0.1 (%), Fe 200 ( $\gamma$  %), Ca 500 (%),

B5(%), Cu 1(%), Mn 100(%), Zn 200(%), Ga 1(%), Co 1(%), Mo 5(%), then 13.7 kg of biotin and 600 mg of yeast fungi self-digestible materials were added to 1,000 cc of this solution, and the pH was adjusted to 6.8 to 8.5 is used as the basal medium. At that time, the constituents are partially substituted with natural substances, depending on the circumstance. The bacteria are cultured for 48 to 72 hours at 25±7°C, under aerobic or anaerobic (facultative anaerobic), and light or dark conditions.

(c) Mass production of above-mentioned bacteria

Although constituents can be partially substituted with natural substances, the respective isolation or proliferative culture medium is used. A large amount of thermophilic fibrinolytic bacteria are cultured anaerobically, or facultative anaerobically by a single step continuous fermentation system, and moreover, a large amount of Rhodospirillaceae are cultured by a multistep circulation-type continuous fermentation system at a rate of 300 to 1,000 L/day.

**Culturing of aerobes, such as Actinomycetes**

(a) Culturing of Actinomycetes

Although difficult generally speaking, Actinomycetes has an important function, along with the other microorganisms, for decomposing various organic substances, and in particular, cellulose, lignin, and the like which are difficult to decompose, and producing humus under fertility of soil. Moreover, it is seen that it is important in the sense of microflow control through the production of biomaterials.

Actinomycetes used in the invention is primarily *Actinomycetes melanoporus*. Culturing of this bacterium is performed by using a Krainsky

(1914) synthetic culture medium consisting of 0.05 g of ammonium chloride, 0.05 g of potassium dihydrogen phosphate, 2.0 g of fibrin, and 100 cc well water or tap water, and maintaining the temperature for 1 to 2 weeks at 27±3°C.

(b) Culturing of filamentous bacteria and yeast fungi

Although filamentous bacteria and yeast fungi are general classifications as a matter of convenience and practical use, both of these belong to the phylum Eumycetes in terms of a systematic taxonomy.

Filamentous bacteria are entrusted to the decomposition of organic substances, such as vegetable remains, which is related to the fertilization of soil. It is thought that they primarily act in the initial step of decomposition.

Next, the function of yeast fungi in soil is often unclear. However, a considerable number of yeast fungi exist in soil and, and their [illegible] with other microorganisms that compete with the minor growth factor they possess, activity in soil, and the like are highly anticipated with future research.

Culturing of filamentous bacteria and yeast fungi is performed on a Czapek Dox (1910) culture medium containing 2 g of sodium nitrate, 1 g of potassium dihydrogen phosphate, 0.5 g of potassium chloride, 0.5 g of magnesium sulfate ( $MgSO_4 \cdot 7H_2O$ ), 0.01 g of ferrous sulfate ( $Fe SO_4 \cdot 7H_2O$ ), 30 g of sucrose (suitable), and 1,000 cc of distilled water; 15 g of agar added as a solid medium.

In this invention, filamentous bacteria, such as *Mucor fragilis*, *Aspergillus* [transliteration], *Penicillium* spp., and *Trichoderma*, are isolated

in soil or compost, and yeast fungi, such as Hansenula, Torula, Endomyces, and Saccharomyces, are isolated therein.

(c) Culturing heterotrophic bacteria (putrefying bacteria)

As with decomposition of sugars, specific bacteria that break /171 down proteins and transform ammonia are rare, and are generally facultative on most bacteria. In this invention, aerobic *Bacillus subtilis* group bacteria are utilized.

Culturing of *Bacillus subtilis* group bacteria is performed in a Waksman (1922) culture medium of 1 g of glucose, 0.5 g of potassium dihydrogen phosphate, 0.2 g of magnesium sulfate ( $MgSO_4 \cdot 7H_2O$ ), trace ferrous sulfate ( $Fe_2(SO_4)_3 \cdot 9H_2O$ ), 0.025 g of egg white (powder), and 1,000 cc distilled water, at a pH of 7.2, and this bacteria group is proliferated aerobically.

(d) Mass production of above-mentioned aerobic bacteria

A culture medium diluted 10- to 20-fold is inoculated with the above-mentioned aerobic bacteria subjected to an isolated or collected culturing with a crude syrup, sterilized air is introduced into this using an 800 to 1,000 L/day, batch device, and a large amount is cultured under aerobic conditions.

An example of crude syrup constituents are as follows, but part of the nitrogen source or phosphorus is added, as needed.

Relatively superior bacteria may be propagated inexpensively and economically in the culturing of various aerobic bacteria.

Constituents of crude syrup

Crude protein	10.0%
Soluble nitrogen-free material	62.1%
Crude ash	
Potassium	3.67%
Calcium	0.74%
Magnesium	0.35%
Sodium	0.16%
Chlorine/sulfur	Tiny amount
Phosphorus	0.08%

Vitamins

Vitamin B <sub>1</sub>	0.4 mg%
Choline	860.0 mg%
Pantothenic acid	18.9 mg%
Niacin	20.0 mg%
Riboflavin/pyridoxine ratio	Large
Vitamins C, E, etc.	Small
Moisture	26.0%
Total digestible ameliorants	54.0%

**Addition of special organic nitrogen sources, vitamins, and minor growth factors**

An amazing number of bacteria on the order of  $\times 10^7$  to  $\times 10^9$  are present in a good-quality plowed layer, as in a paddy field or farmland. Only 15% of these bacteria are able to grow in sugar and inorganic salts. The majority of the bacteria require some form of amino acid, vitamin,

VGF (unidentified growth factor).

Both thermophilic fibrinolytic bacteria and Rhodospirillaceae are no exception to this. Supposing these bacteria are depleted, continuous culturing of thermophilic fibrinolytic bacteria becomes impossible and propagation of Rhodospirillaceae is suspended, so an abnormal fermentation occurs.

Therefore, the first minor growth factor is VGF- $\alpha$  and the latter is VGF- $\beta$  (alias: Gloucester). These new growth factors were discovered by the inventors of this invention. 40 ppm or more of VGF- $\alpha$  and 0.5 ppm or more of VGF- $\beta$  are respectively used for culturing.

Moreover, according to the reasons described above, for general bacteria which are effective in soil, the following minor nutrients are added to the soil activator of this invention.

Vitamin B <sub>1</sub> (thiamine)	1.00	ppm	or	more
Vitamin B <sub>2</sub> (riboflavin)	5.00	"	"	"
Nicotinic acid	800	"	"	"
Vitamin B <sub>6</sub> (pyridoxine)	0.40	"	"	"
Pantothenic acid	400	"	"	"
Folic acid	0.20	"	"	"
Choline	10.0	"	"	"
Biotin	0.20	"	"	"
Vitamin B <sub>12</sub> (cobalamin)	0.05	"	"	"
Paraamino benzoic acid	5.00	"	"	"
Corn steep liquor (CSL)	0.01%	"	"	"
Defatted soybean hydrochloric acid hydrolysate	0.03%	"	"	"

The physical properties of vermiculite include its large potassium content, high porosity, excellent moisture absorption and holding ability, good drainage and air circulation, and particularly powerful substitutability of bases, etc. But not only do the calcium carbonate rock powder, calcium ions and magnesium ions become nutrient sources for effective bacteria on soil, such as thermophilic fibrinolytic bacteria, they are useful for adjusting the hydrogen ion concentration in soil and creation of a granulated soil structure and for making conditions for a satisfactory soil environment.

Therefore, the particle sizes of the excipient and soil activator are determined according to their physical properties, their compounding ratios, which are from 10% to 50% of the calcium carbonate rock powder, depending on the method of using arable land, the properties of the soil, the type of cultivated plant, or the like, the type of spreader, etc. Lastly, the form of the soil activator, such as powdered, pellet-shaped, or pearl-shaped, is determined under an integrated determination by considering the contamination, preservation, process control, economics, deterioration and extermination of harmful bacteria and inoculum thereof.

Therefore, in this invention, as described above, in addition, to an activator composed mainly of the organic nitrogen source, vitamins, minor growth factors, and the like, as well as the vermiculite and calcium carbonate rock powder, upon stirring and mixing these well and using a culture medium, a product with a predetermined shape is obtained from an aerobic crude syrup culture of a concentrated microbial cell fluid

is obtained by adding a respectively conforming natural polymer and flocculant, Actinomycetes, filamentous bacteria, yeast fungi, and heterotrophic bacteria, for the anaerobic and facultative anaerobic culturing of bacteria useful for aging and humifying organic substances in the soil, i.e., thermophilic fibrinolytic bacteria, Rhodospirillaceae, etc.

An example of compounding raw materials is as follows.

Compounding Ratio of Raw Materials

(in 1,000 g of calcium carbonate rock powder)

Conc. thermophilic fibrinolytic bacteria microbial cell fluid	0.2 g
Conc. Rhodospirillaceae microbial cell fluid	0.5 g
Crude syrup of Actinomycetes, filamentous bacteria, yeast fungi and heterotrophic bacteria	5.0 g
Culture fluid	55.0 g
VGF- $\alpha$	15.0 mg
VGF- $\beta$ (alias: Gloucester)	1.2 mg
Vitamin B <sub>1</sub>	1.2 mg
" " B <sub>2</sub>	5.5 mg
Nicotinic acid	830.0 mg
Vitamin B <sub>6</sub>	0.5 mg
Pantothenic acid	420.0 mg
Folic acid	0.3 mg
Choline	12.0 mg
Biotin	0.2 mg
Vitamin B <sub>12</sub>	0.1 mg
Paraaminobenzoic acid	7.0 mg
Corn steep liquor (CSL)	0.3 mg
Defatted soybean hydrochloric acid hydrolysate	0.7 mg
Vermiculite	200 g
Carbon rock powder	1,000 g

The following merits may be cited as the superior advantages of this invention as such.

(a) Culturing bacteria which are effective on soil, such as thermophilic fibrinolytic bacteria, Actinomycetes, filamentous bacteria, Rhodospirillaceae, yeast fungi, or heterotrophic bacteria, and improving

the density of the bacteria by adding them to the soil artificially is currently one remarkably effective method for an agricultural soil preparation in Japan.

(b) Whether or not such an artificial inoculating method is successful and whether the conditions for fixing and activating the bacteria are established, the vermiculite and the calcium carbonate rock powder of the excipient scattered in large amounts simultaneously play a role for enriching the [illegible] of sophisticated microorganisms, such as the drainage, circulation, absorption of moisture, holding of water, creation of a granulated structure, and adjustment of the hydrogen ion concentration, and at the same time, for improving the conditions for the soil environment for cultivated plants.

(c) The addition of various minor nutrients required of microorganisms in the soil and the propagation of Rhodospirillaceae and yeast fungi /173 are performed with good succession in a soil microorganism system, and the genuine humification of organic substances in the soil is performed reliably and rapidly.

(d) Moreover, the preservation, scattering, and the like of a solid inoculum are easy and reliable by selecting the form thereof, such as powdered, pellet-shaped or pearl-shaped. Moreover, the validity of the inoculum of the soil activator of this invention is maintained for several years in places with relatively good preservation conditions, such as places with low humidity, cold and dark places, etc.

The superb advantages of the soil activator will be further demonstrated in a few practical examples in which it is applied according to this invention.

### Practical Example 1

Compost is a raw material with the maximum integrated effects for "soil preparation." The soil activator of this invention also exhibits a superb advantage for aging compost.

60 kg of a powdered soil activator (vermiculite: calcium carbonate rock powder = 20:100) and moisture were added to 1,000 Kg of rice straw and temporarily heaped for about 10 days. Next, ammonium sulfate or urea equivalent to 1.2 kg of nitrogen was scattered thereon to make a main pile while sprinkling water on it and lightly trampling it properly. This is repeated once halfway through the procedure. The compost is fermented completely in 45 days.

The compost is aged well to the extent that the rice straw can be torn into pieces readily and the carbon rate is 17.3.

Then, as a result of preparing controls with and without adding 50 kg of a mixture of vermiculite and calcium carbonate rock powder (ratio=20:100) instead of the soil activator of this invention, and applying them concurrently in the same way as the method for applying a soil activator, the compost in the first control was semi-aged and no compost was verified in the latter control. Moreover, the carbon rate of the first control was 31.8, while that of the latter was 37.2.